Ind. J. Physiol. Pharmac., Volume 33, Number 2, 1989

THE SYMPATHETIC NEURO-MELANOPHORE TRANSMISSION IN A FRESH-WATER INDIAN MAJOR CARP, *LABEO ROHITA* (HAM.)*

SHASHI PATIL AND AJAI K. JAIN**

Pigment Cell Biology Unit,
School of Studies in Zoology,
Jiwaji University, Gwalior - 474 011

(Received on September 28, 1988)

Summary : Adrenaline was effective in aggregating the melanosomes both in innervated as well as denervated melanophores. Isotonic KCl could induce pigment aggregation only in innervated melanophores. Adrenaline-and K^+-induced pigment aggregation response in these melanophores was blocked by phentolamine: propranolol failed to do so. It is suggested that the chromatic nerves in the fish, *Labeo rohita* are adrenergic and via post-synpatic α-adrenoceptors, control the melanosome aggregation.

Key words : melanophores, drug effects, neurotransmission, alpha receptor, adrenergic fibres

INTRODUCTION

Mechanisms of colour change are complex in teleosts in which humoral factors from the hypothalamus, pituitary gland and the pineal gland and autonomic nervous system combine to regulate not only the motile activities of specialized integumentary coloured cells, the chromatophores (physiological colour change) but also the net amount of pigmentary materials within them (long term morphological colour change) (1). Neural regulation takes a role in adapting fish more rapidly to the environment. The hormonal means may have evolved to control chromatophore motility slowly, since the process inevitably includes the gradual increase or decrease of their titers in the blood. Such dual regulation in *Labeo rohita* (Ham.), a fresh-water Indian major carp has been demonstrated in our laboratory recently (2).

*Support was provided by a Research Grant D-25 7 (13627) (SR-I) from U.G.C., NEW DELHI.

**Corresponding Author

The aim of this investigation was to gain precise information pertaining to the nature of innervation of melanophores and receptors therein. Attention has also been paid to *in vitro* analysis of pigment aggregating action of K^+.

MATERIALS AND METHODS

Labeo rohita (Ham.), of either sex obtained from Government Fish Farm, Datia was used. Fish at a fiingerling stage (body lengths, 5 to 7 cm) were selected. They were maintained as described elsewhere (2).

Dermal melanophores designated as type 2 for convenience and distributed in the intermediate region between the margin and the centre of the skin tissue (which remains attached to the posterior or exposed part of the scale when the latter is detached from the fish body) were found suitable for the present investigation.

Experiments were conducted on the scales isolated from the antero-dorsal part and fastened epidermis side down to the undersurface of the coverglass mounted on a trough made of perspex sheet containing physiological saline (composition (mM) : NaCl 128, KCl 2.7, $CaCl_2$ 1.8, Glucose 5.6, HEPES-NaOH buffer 10.0, pH-7.4). The effect of drugs on the response of certain group of dermal melanophores were studied with a light microscope (Carl Zeiss 100X) and were evaluated according to Melanophore Index (M. I.) as introduced for amphibians (3), where 1 stands for maximal aggregation of melanosomes and 5 stands for a state of their complete dispersion. A change of one unit or more in the mean M.I. was arbitrarily designated as significant (4).

Drugs used were adrenaline tartarate (Harson Laboratories, Baroda), reserpine (Serpacil) and phentolamine mesylate (Hindustan CIBA GIEGY Ltd., Bombay) and propranolol hydrochloride (CIPLA, Bombay). A fresh injection fluid or a concentrated stock solution of a drug was diluted with physiological saline or K^+–rich saline (in which Na^+ was totally replaced by K^+) or Ca^{2+}–free saline (made by withdrawing $CaCl_2$ and adding disodium EDTA, 0.2mM). All solutions were applied to the isolated scale in the trough so as to bath the skin tissue of the scale and in order to change solution in the trough, it was once drained thoroughly by an outflow pipette connected with another solution introduced through an inflow siphon. All values are given as means $\pm$ S.E.M. All experiments were performed at room temperature—20–22°C (slow drifts, $< \pm$ 1.2°C over several hr).

In order to get denervated but otherwise functional melanophores, the fish were given a single injection of reserpine (10 μg/g, ip). Scales for experimentation were isolated 2-5 hr post injection.

RESULTS

Melanophores in the scales isolated from the fish totally lost the capacity to aggregate their melanosomes in response to K^+—rich saline but they responded to adrenaline with a rapid melanosome aggregation (Fig. 1).

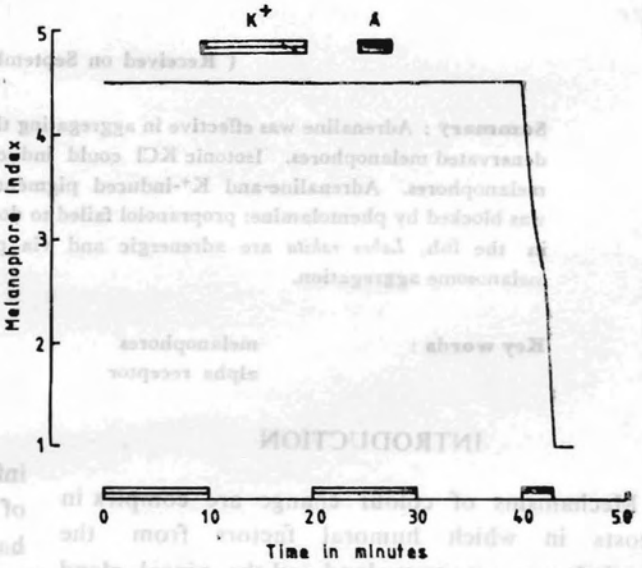

Fig. 1 : Ineffectiveness of K^+ but effectiveness of adrenaline (A) (5×10^{-5}M) to arouse rapid melanosome aggregation (decreased melanophore index) in melanophores obtained from a reserpinised fish. (n=5; SEM values between 0.04 and 0.10 M.I.-unit).

Type 2 melanophores in the scales from antero-dorsal part (See Methods) had M.I. less than 2 or even fully aggregated (M.I.=1). At this stage they appear very dark (black). Gradually they start showing dispersion and in approximately 20 min all melanophores attain their maximal dispersion showing brownish tinge with homogenous melanosome distribution, which served as the starting point for further investigations. The pigment-dispersing state persisted unchanged for at least 2 hr. A constancy in the shape, size, and melanin content (visual estimation) rendered the type 2 melanophores suitable for experiments

The melanophores in isolated scales not only maintained their dispersed state in the physiological solution but in response to K^+ or adrenaline they achieved pigment aggregation. Fig. 2 illustrates typical responses to K^+–rich saline (133 mM) and its blockade by phentolamine (10^{-4}M). High K^+ solution was able to produce complete melanosome aggregation within 5 min. Recovery from pigment aggregation proceeded rapidly after the withdrawal of K^+ stimulation and melanophores attained initial state of pigment dispersion within 15 min. High K^+ solution could repeatedly induce comparable melanosome aggregation.

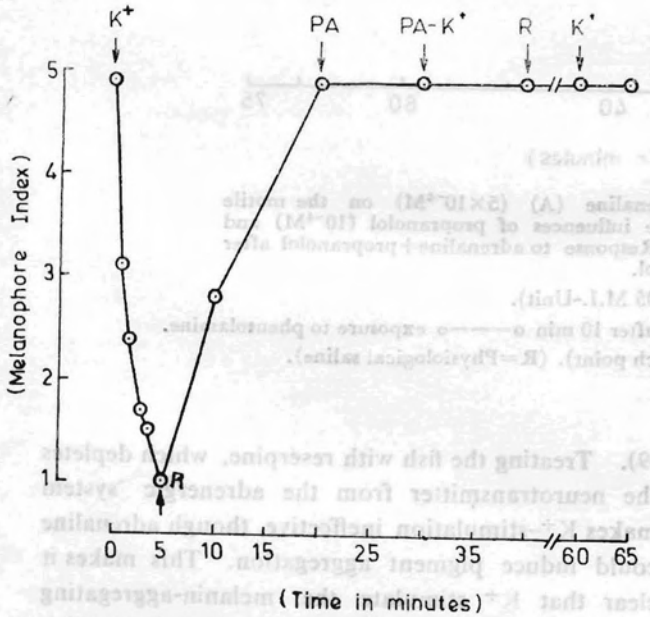

Fig. 2 : Response of innervated melanophores of *Labeo rohita* to the K^+ rich (133 mm) saline solution before (at K^+) and after phentolamine (PA 10^{-4}M) on the melanin-aggregation (at PA+K^+).
(n=5; SEM values between 0.02 and 0.1 M.I.-unit. R=Physiological saline).

The K^+-action was not blocked by propranolol (10^{-4}M), a β-adrenoceptor blocking agent. Fig. 3 indicates a typical graded-aggregation response to increasing concentrations of adrenaline on the same melanophore population (5 in number) from an isolated scale of a fish.

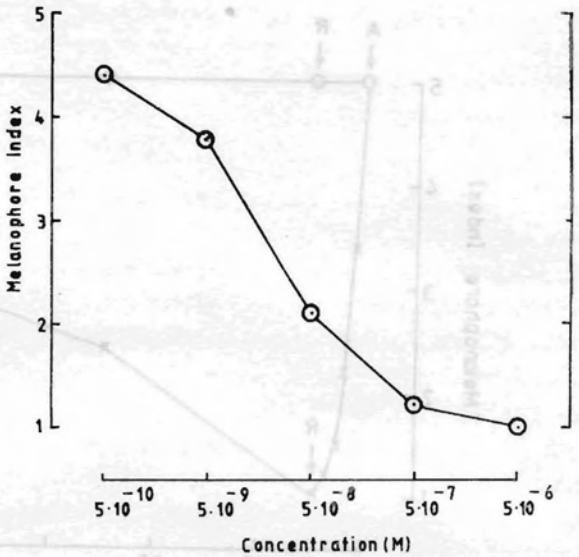

Fig. 3 : A typical concentration-response curve for melanosome-aggregating effect of adrenaline, (applied for 10 min). Each point is the mean of five different estimates.

The melanin aggregation induced by adrenaline was completely antagonized (Fig 4) by phentolamine, (10^{-4}M; exposure time, 10 min). However propranolol (10^{-4}M) had no blocking action on the aggregation response to adrenaline (10^{-6}M). Withdrawal of Ca^{2+} had practically no influence either on the pigment-aggregating action of adrenaline or on the recovery from this effect. Loss of response to K^+–rich saline and pigment aggregation in response to adrenaline (5×10^{-5}M; Fig. 1), blockade of response to adrenaline by phentolamine but not by propranolol were similar in denervated and innervated melanophores. After K^+ stimulation when the scale was reimmersed in the physiological saline for a long period (90 min), a profound dispersion of melanosomes was observed, the phenomenon being more pronounced in the innervated than in denervated preparations.

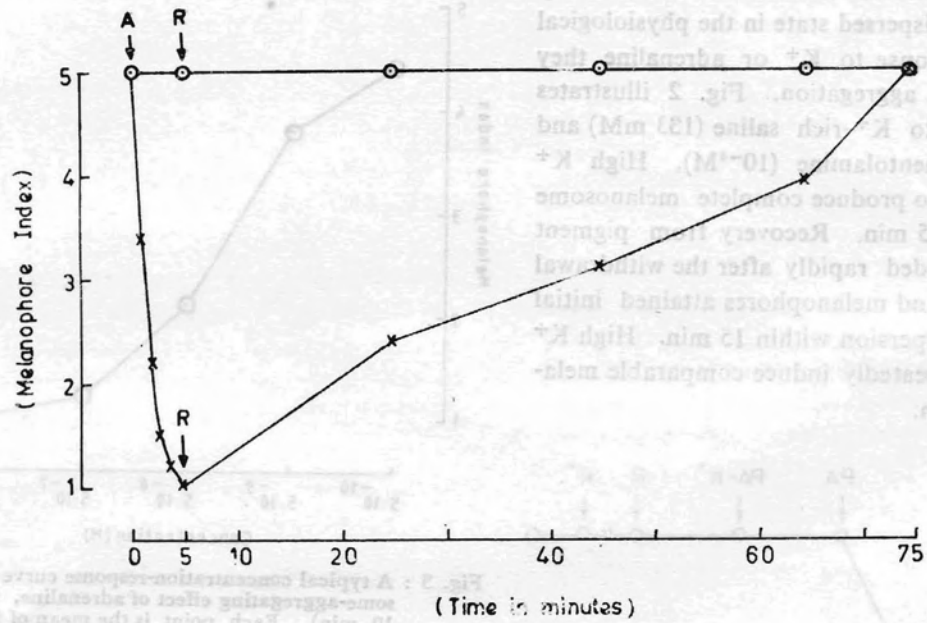

Fig. 4 : Melanin aggregating action of adrenaline (A) (5×10^{-6}M) on the motile melanophores of *Labeo rohita* and the influences of propranolol (10^{-4}M) and phentolamine (10^{-4}M) on this action. Response to adrenaline+propranolol after 10 min x———x exposure to propranolol.

(n=5; SEM values between 0.03 and 0.05 M.I.-Unit).

Response to adrenaline+phentolamine after 10 min o———o exposure to phentolamine.

(n=5; SEM, value 0.05 M.I.-unit at each point). (R=Physiological saline).

DISCUSSION

Our prime concern in studying K^+–action on melanophores in *Labeo rohita* was to gather information about the nature of the chromatic fibres. K^+ always induced a rapid and reversible pigment aggregation in innervated melanophores. The reaction time observed was comparable to that reported for other fish species (1, 8). Secondly, in most of the denervated melanophores, the high K^+ stimulation could not induce pigment aggregation indicating that K^+ acts indirectly through a neuronal mechanism. Thirdly, the pigment aggregation by K^+ was not blocked by propranolol, showing that it is independent of β-adrenoceptors. Adrenaline could induce pigment aggregation in denervated melanophores. Adrenaline acts on the nerve endings as well as directly on the melanophore themselves (9). Treating the fish with reserpine, which depletes the neurotransmitter from the adrenergic system makes K^+–stimulation ineffective, though adrenaline could induce pigment aggregation. This makes it clear that K^+ stimulate the melanin-aggregating adrenergic terminals to liberate the transmitter which then acts to aggregate pigment in melanophores. This is in agreement with reports involving related fish species (5, 7, 10-13).

Yamada *et al.* (14) looked into the possibility of adrenergic innervation to melanophores in scales of *Oryzias latipes* using ^3H norepinephrine by light microscopic autoradiography. In their work K^+ caused a considerable reduction in the labelling of varicose fibres, which was accompanied by aggregation of melanosomes within melanophores.

One step ahead to it, Kumazawa and Fujii (15) using radiolabelled norepinephrine and adenosine clearly demonstrated the actual liberation of the amine and adenine derivatives from the neural elements in response to K^+ stimulation. As this release was significantly reduced by bretylium they concluded that upon stimulation of the nerve, both the true transmitter, norepinephrine and the co-transmitter, adenine nucleotides, are liberated for the control of chromatophore movements.

From the present results we speculate that high $(K^+)_o$ action initially induces a presynaptic membrane depolarization, leading to an increase in Ca^{2+} influx, finally resulting in release of the neuro-transmitter from the adrenergic nerve terminals. Such a mode of action of K^+ in systems other than the pigment cells is supported from the literature (16, 17). For the pigment cells, it has, however, been reported by Kumazawa and Fujii (18) for the first time.

Further, in the present observations, the aggregation of melanosomes due to K^+ depolarization of the melanophores was completely inhibited by phentolamine, an alpha-adrenoceptor antagonist which also inhibited the pigment aggregating action of exogenous adrenaline. Propranolol was ineffective against K^+ or adrenaline. These results provide a strong basis that neurotransmitter responsible for pigment aggregation is noradrenaline and that the cellular receptors involved are α-adrenoceptors,

Adrenaline induced melanosome aggregation was not influenced by the Ca^{2+} deprivation. The response of innervated melanophores evoked by the adrenaline was dose related between concentrations 5×10^{-9} and 5×10^{-6}M (Fig. 3). It was interesting from a standpoint of comparative physiology of chromatophores that the above concentrations of adrenaline agreed closely with the threshold concentration and the maximally effective reported concentration in other fish species (11, 19 & 20).

Practically all the Indian teleosts tested for the chromomotor-colour changes (2, 21, 22) including one under the present study change their colour quickly when transferred to illuminated black background by dispersing their integumentary melanophores. This agrees with the concept of neural regulation of melanosome movements described for *Tilapia* (15). Further the contention of spontaneous release of small amount of ATP in the bathing medium from isolated scale/split-fin preparations during unstimulated period which is responsible for maintenance of melanophores in dispersed condition seems to be true for our findings on the *Labeo rohita* and other Indian teleosts (22).

Kasukawa *et al.* (23) while working on *Chrysiptera cyanea* explained the extraordinary rapid rate of motility of its melanophores. The melanophores from *Chrysiptera cyanea* exhibit a very rapid motility (23) since they are small (about 50 to 80 μm in diameter) and the diameter of the cellular processes through which the melanosome travel is fairly large. In *Labeo rohita*, the melanophores are small in diameter but the cellular processes are not so thick as in the fish *Chrysiptera*, so the motility of the melanophores though rapid is not so high as in *Chrysiptera*.

We conclude that in *Labeo rohita* the melanophores are innervated by pigment-aggregating fibres which are adrenergic and melanophores have α-adrenoceptors. Fujii *et al.* (25) put the loach, *Misgurunus anguillicaudatus* to a group of fish in which chromatophores are primarily under the control of endocrine systems. In the present study the response to K^+—stimulation and adrenaline were found to be very rapid, bringing about full aggregation of melanophores, confirming a neural control in *Labeo rohita* (See, 2).

ACKNOWLEDGEMENTS

We thank Dr. J. Bahadur, Professor and Head, School of Studies in Zoology, Jiwaji University, Gwalior, for providing the necessary laboratory facilities and encouragement.

REFERENCES

1. Fujii R and Oshima N. Control of chromatophore movements in teleost fishes. *Zool Sci* 1986; 2 : 13-47.
2. Bhatnagar K. Some aspects of colour changes in the common carp, *Cyprinus carpio* and a major carp, *Labeo rohita*. (Dissertation) Gwalior Jiwaji University, 1985; 45 p.
3. Fange R. Pharmacology of poikilothermic vertebrates and invertebrates. *Pharmacol Rev* 1962; 14 : 281-316.
4. Abbott FS. The effects of certain drugs and biogenic substances on the melanophores of *Fundulus heteroclitus L. Can J Zool* 1968; 46 : 1149-61.
5. Fujii R. Mechanism of ionic action in the melanophore system of fish I. Melanophore concentrating action of potassium and some other ions. *Annot Zool Japon* 1959; 32 : 47-58.
6. Ishibashi T. Action of potassium ion on fish melanophore with some considerations of the characteristic of the isolated fish scale. *Zool Mag* 1950; 59 : 336-43.
7. Iga T. Variation in response of scale melanophores of teleost fish, *Oryzias latipes*, to potassium ions. *J Sci Hiroshima Univ* 1975; 26 : 23-35.
8. Iga T. The mode of action of potassium ions on the leukophores of a freshwater teleost, *Oryzias latipes*. *The J Exptl Zool* 1978; 205 : 413-22.
9. Fujii R. Chromatophores and Pigments. In : Hoar WS, Randall DJ, eds. Fish Physiology; vol. 3, New York : Academic Press, 1969 : 307-53.
10. Grundstrom N, Karlsoon JOG and Andersson RGG. The control of granule movement in fish melanophores. *Acta Physiol Scand* 1985; 125 : 415-21.
11. Iga T, Matsuno A. Scale melanophores of *Zacco temmincki*. A preparation suitable for physiological or pharmacological studies on fish melanophores. *Zool Mac* 1980; 89 : 227-34.
12. Iwata KS, Watanabe M and Nagao K. The mode of action of pigment concentrating agents on the melanophores in an isolated fish scale. *Biol J Okayama Univ* 1959; 5 : 195-206.
13. Nomoto S and Yamada K. Effects of monovalent cations on denervated fish melanophores, with special reference to the action of lithium ions. *J Sci Hiroshima Univ* 1983; 31 : 107-15.
14. Yamada K, Miyata S and Katayama H. Autoradiographic demonstration of adrenergic innervation to scale melanophores of a teleost fish, *Oryzias latipes*. The J Exptl Zool 1984; 229 : 73-80.
15. Kumazawa T and Fujii R. Concurrent releases of norepinephrine and purines by potassium from adrenergic melanosome-aggregating nerve in *Tilapia*. *Comp Biochem Physiol* 1984; 78 : 263 66.
16. Garicia AG, Kirpekar SM and Pascual R. Release of noradrenaline from Cat spleen slices by potassium. *Br J Pharmac* 1978; 62 : 207-11.
17. Kirpekar SM and Wakade AR. Release of noradrenaline from the cat spleen by potassium. *J Physiol (London)* 1968; 94 : 595-608.
18. Kumazawa T and Fujii R. Fate of adenylic co-transmitter released from adrenergic pigment-aggregating nerve to Tilapia melanophore. *Zool Sci* 1986; 3 : 599-603.
19. Fujii R and Miyashita Y. Receptor mechanisms in fish Chromatophores-I. Alpha nature of adrenoceptors mediating melanosome aggregation in guppy melanophores. *Comp Biochem Physiol* 1975; 51 : 171-178.
20. Nagahama H. Action of potassium ions on the melanophores in an isolated fish scale. *Jap J Zool* 1953; 11 : 75-85.
21. Jain, AK. Studies on the colour change mechanism in a fresh water fish. (Thesis), Sagar University of Saugar, 1977; 258 p.
22. Singh D, Bhargava HN and Jain AK. On background related chromatic response in the cave fish, *Nemachilus Evazardii* (Day). *J Anim Morph Physiol* 1984; 31 : 203-9.
23. Kasukawa H, Sugimoto M. Oshima N and R Fujii Control of chromatophore movements in dermal chromatic units of blue damselfish-I. The Melanophore. *Comp Biochem Physiol* 1985; 81 : 253-7.
24. Fujii R, Jain AK and Miyashita Y. Note on the nervous control of melanophores of the loach, *Misgurnus anguillicaudatus*. *J Lib Arts & Sci Sapporo Med Coll* 1980; 21 : 61-3.